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## RAT CRUSH STUDY

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TITLE AND SUBTITLE: Effect of Hyperbaric Oxygen on Crush Injury in the Rat

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## ABSTRACT:

When work commenced on this project according to the protocol, a device was constructed to drop a multi-kilo weight through a distance of up to one meter so that it would produce a crushing injury to a rat tail. When this was initially tried, it was found that injury was not predictable, as sometimes vertebrae would be crushed, injuring the ventral artery, causing a complete loss of the tail. If the ventral artery were not damaged, the tail survived without appreciable injury. It was also noted that tissues of the tail contained mostly tendon and very little muscle. Following this, the crushing agent was modified to traumatize a musculocutaneous

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flap which was raised on the rat's back. Again, a uniform and replicable crush injury could not be produced. An Instron machine was then modified to apply a slow, squeezing motion to the flap, but the machine proved to have inadequate power to produce a lasting injury. The traumatizing apparatus was modified once again, and this time a rabbit ear was used. Again, little damage occurred, even when prodigious amounts of force were delivered. Because of the abundance of cartilage and very little muscle, this is also not a good model. Finally, a model created by the Department of Plastic Surgery at the University of Utah was tried. Purportedly, this model had been used successfully for a number of years. In it, muscle of the rat thigh was crushed by a dropped weight, the area of injury being 177mm<sup>2</sup>.

This time, damage to the tissue was to be measured using a vital stain, triphenyltetrazolium chloride. This stain determines the difference between viable and dead tissue.

However, following crush injury, when rats were injected with the dye, the staining was quite irregular, and it was not possible to determine the difference between the treated and the control group. One of the difficulties was dissecting out precisely that area of the thigh which had been crushed.

We now are investigating other methods of quantifying the damage in the crushed region, which may include electron microscopy of the damaged area, assay of superoxide dismutase in the damaged area, quantitative evaluation of vascular permeability using Evans blue fluorescence, and using electron spin resonance to determine free radical formation.

Once the model has been perfected and is reproducible, any of these techniques can be used. This project continues with the first goal to achieve a reproducible model. After Air Force funding has been exhausted, work will be completed using funding from other sources. A report will be delivered to the Air Force at that time.

SUBJECT TERMS: Crush Injury, HBO

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**ABSTRACT (Brief form)**

Protocol called for a device to drop a multi-kilo weight up to one meter to produce crush injury to a rat tail. However, the injury was not predictable. If the ventral artery were undamaged, the tail survived without appreciable injury. The traumatizer was modified to crush a musculocutaneous flap raised on the rat's back. Replicable crush injury could not be produced. Next, an Instron machine was modified to apply a slow, squeezing motion to the flap, but force proved inadequate to produce lasting injury. The traumatizing apparatus was again modified, and this time a rabbit ear was used. Again, because of the abundance of cartilage and lack of muscle, little damage occurred. Finally, a model created by the University of Utah was tried. Rat thigh muscle was crushed by a dropped weight, the area of injury being 177 mm<sup>2</sup>. Damage was measured using triphenyltetrazolium chloride to differentiate viable and dead tissue. However, staining was quite irregular, and the difference between the treated and the control group was unclear.

Other methods of quantifying damage in the crushed region may include electronmicroscopy, assay of superoxide dismutase, measuring vascular permeability using Evans blue fluorescence, and using electron spin resonance to determine free radical formation. Any of these techniques can be used with a good model. This project continues with the first goal to achieve a reproducible model. After Air Force funding is exhausted, work will be completed using other funding. A report will then be delivered to the Air Force.